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Prevalence and molecular characterization of *Cryptosporidium* species among herds in selected Local Government Areas of Kaduna State, Nigeria

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Abstract

Cryptosporidiosis is a neglected tropical zoonotic disease caused by a protozoan parasite of the genus *Cryptosporidium*. The aim of the study was to determine the occurrence of *Cryptosporidium* antigen and species of the parasite in livestock and dogs in sedentary Fulani herds in the selected Local Government Areas (LGAs) of Kaduna State, Nigeria. Seven hundred and fifty faecal samples (240, 180, 240 and 90 from cattle, sheep, goats and dogs, respectively) were collected. Faecal samples were screened with a commercial Enzyme-linked immunosorbent assay (ELISA) kit and those positive for *Cryptosporidium*, were subjected to nested Polymerase Chain Reaction (nPCR). Direct sequencing of the nPCR products were carried out to identify the species. The overall prevalence of *Cryptosporidium* antigen in faeces was 4.2% (10/240), 1.7% (3/180), 1.1% (1/90) and 0.4% (1/240) for cattle, sheep, dogs and goats respectively. Significantly higher prevalence of *Cryptosporidium* species antigen ($p = 0.02$) was observed in sheep ≤ 6 months of age than those above 6 months age. Dogs passing out loose/watery faeces had significantly higher prevalence of the infection ($p=0.05$) than those with firmly formed faeces. Among the species detected in this study, *Cryptosporidium andersoni* (42.9%) was most prevalent, followed by *C. muris* (21.4%) and *C. parvum* (21.4%), and the least was *C. hominis* (14.3%). *Cryptosporidium parvum, C. hominis, C. andersoni* and *C. muris* were detected in cattle, *C. andersoni* from sheep and goats, and *C. muris* from dog faeces. The presence of *C. parvum* and *C. hominis* in cattle in this study suggests that the dairy cattle in these LGAs have high potential for the transmission of *Cryptosporidium* to humans. Therefore, inhabitants of these LGAs should be informed and educated on the need for improved sanitary measures during milking these animals and the need for adequate pasteurization of milk before consumption.

Keywords: Cattle, Cryptosporidiosis, *Cryptosporidium* antigen, Dogs, Goat, Nested PCR, Sheep

Introduction

Cryptosporidium is an enteric protozoan parasite that causes diarrhoea and other clinical symptoms in many mammals including humans (Xiao, 2010). This protozoan is included in the World Health Organization (WHO) "Neglected Tropical Diseases Initiative" (Savioli *et al*., 2006) and *Cryptosporidium* is

considered the second most common cause of diarrhoea and death in children in developing countries after rotavirus (Kotloff *et al*., 2013). About 33 *Cryptosporidium* species have been recognized and of these, more than 17 have been identified in humans (Ryan *et al*., 2016). By far the most common species reported in humans worldwide are *C. parvum* and *C. hominis* (Xiao, 2010). Cattle are the mammalian species commonly infected with *Cryptosporidium*, and preweaned calves are considered the most important reservoirs for zoonotic infections. *Cryptosporidium bovis*, *C. ryanae* and *C. andersoni* are the commonly encountered species affecting cattle in Nigeria (Ayinmode & Fagbemi, 2010; Maikai *et al*., 2011). A large number of studies have suggested that *C. parvum*, *C. bovis*, *C. andersoni*, and *C. ryanae* are the most common species infecting cattle, (Trout & Santin, 2008). Dairy cattle have been considered to be a major host for *C. parvum*, of which pre-weaned calves are frequently infected with this species (Trout & Santin, 2008).

Some studies have revealed that sheep are more frequently infected by other apparently host-adapted *Cryptosporidium* genotypes, mostly *C. bovis* (Elwin & Chalmers, 2008). Other *Cryptosporidium* species that affect sheep and goats are *C. parvum*, *C. hominis*, *C. xiaoi*, *C. andersoni*, *C. fayeri* and *Cryptosporidium* pig genotype II (Fiuza *et al*., 2011). Dogs have also been suggested to be a significant source of human cryptosporidiosis and can be naturally infected with *C. canis*, *C. parvum*, and *C. meleagridis* (Olabanji *et al*., 2016).

Studies on cryptosporidiosis have been reported in Jos, Abeokuta, Sokoto, Maiduguri, Kebbi State, Abuja and Kwara State (Pam *et al*., 2013; Akinkuotu *et al*., 2014; Faleke *et al*., 2014; Adamu *et al*., 2015; Danladi and Ugbomoiko, 2015; Olabanji *et al*., 2016; Ola-Fadunsin *et al*., 2022). The prevalence of *Cryptosporidium* species has been reported in humans, piglets, cattle, birds and raw vegetables within the study area (Maikai *et al*., 2009; 2011; 2012; 2013; Bamaiyi *et al*., 2013; Okojokwu *et al*., 2016a; 2016b). The prevalence of this parasite in sedentary Fulani settlements which provide the populace with fresh nutritive milk is yet to be determined, thus, a need for this work in order to determine the prevalence status in these sedentary Fulani herds and to help provide baseline data for effective disease prevention and control.

The aim of the study was to determine the occurrence of *Cryptosporidium* antigen and species of the parasite in livestock (cattle, sheep and goats) and dogs in sedentary Fulani herds in the selected Local Government Areas (LGAs) of Kaduna State, Nigeria.

Materials and Methods

Study area

The study was carried out in six (6) selected LGAs of Kaduna State, Nigeria; namely, Zaria, Sabon Gari, Giwa, Igabi, Soba and Kudan.

Study design

This was a cross-sectional study in which 240, 180, 240 and 90 faecal samples were collected from cattle, sheep, goats, and dogs respectively in sedentary Fulani herds in selected LGAs of Kaduna State, Nigeria from May to September 2021. Five cattle herds were selected from each of the six LGAs based on inclusion criteria as follows: (1) Settled herds of ≥ 20 cattle capacity (2) Herds which produce and sell raw cow milk for public consumption (3) Herds that rear cattle with other ruminants (sheep or goat) or at least a dog. Based on these criteria thirty cattle herds were selected for this study. Eight cattle in each herd were selected using systematic random sampling. A total of 30, 40 and 15 faecal samples were collected from sheep, goats and dogs respectively in each LGA while samples in each herd were collected based on availability.

Sample collection

About 10g of faeces was collected directly from rectum of randomly selected animals using clean disposable rubber hand glove for each animal, and emptied into sterile sample bottles. All samples were properly labeled and transported on ice packed cold box to the Parasitic Zoonoses Laboratory, Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria.

Laboratory procedures

Detection of *Cryptosporidium* spp. antigen in faeces using an ELISA kit: Faecal samples were screened for the presence of *Cryptosporidium* antigens using a commercial ELISA kit (CaproELISA^{TM}, Savyon^{R} Diagnostics Limited, Israel; specificity 94% and sensitivity, 98.9%). This test was carried out as described by the manufacturers. All test-positive faecal samples were preserved in 2.5% potassium dichromate and stored in a refrigerator at 4°C for further molecular analysis.

Nested PCR: The fast DNA kit for soil (BIO 101, Carlsbad, CA) was used in DNA extraction according to the manufacturer's instruction as described by

Feng *et al*. (2007). A nested PCR protocol based on the amplification of a specific sequence of 18S rRNA gene was used to detect *Cryptosporidium* species (Xiao *et al*., 2000). The method involves the amplification of an approximately 1,325bp-long primary product followed by a secondary amplification of an internal fragment with a length of approximately 830bp. The mentioned gene fragments were amplified with the primer pairs in Table 1 for the first and second rounds of PCR amplification. Then 35 cycles were performed for the primary PCR with denaturation at 92°C for 60 seconds, annealing at 56°C for 60 seconds, and extension at 72°C for 60 seconds. The reaction mixture was initially subjected to denaturation at 95°C for 3 minutes for complete denaturation of the template and the final extension step consisted of incubation at 72°C for 7 minutes. For the secondary PCR, the cycling conditions were identical to the conditions used for the primary PCR except for the annealing temperature that was set at 58°C. Each set of experiments included a positive PCR control consisting of 1µl of specific DNA template (*C. parvum* was used in this case) and a negative PCR control which was the master mix without any *Cryptosporidium* DNA.

Gel Electrophoresis: The amplified products from PCR were detected and verified for size, by running a 1.5 % agarose gel which was stained with ethidium bromide. The gel was viewed under a UV transilluminator (G-BOX) and the band sizes were determined by comparing with the 100bp ladder.

DNA Sequencing of PCR Product: Direct sequencing of the secondary PCR products of the 18S rRNA gene was carried out using an automated DNA sequencer (BigDye Terminator Chemistry) following Sanger's sequencing method to identify the species. Sequencing was performed directionally using forward and reverse sequencing primers. The resulting. ab1 files from the sequencer were exported as FASTA files for sequence analysis. The sequences obtained were compared with published sequences in the GenBank database using Basic Local Alignment Search Tool (BLAST) from the National Center for Biotechnology Information (NCBI, 2023) and computer program CLUSTAL_X 2.

Data analysis

Data from the study were analysed using Statistical Package for Social Science (SPSS) version 20.0 (Standard Version SPSS Inc., Chicago, IL, USA). Chi-square and Fisher's Exact Test were used to test for association between *Cryptosporidium* antigen and factors such as age, sex, breed, consistency of faeces, and body condition score. P values ≤ 0.05 were considered significant.

Results

On analysis of the ELISA results, the overall prevalence of *Cryptosporidium* species antigen in cattle faeces was 4.2% (10/240) (Table 2). The prevalence of *Cryptosporidium* species antigen in sheep and goats was 1.7% (Table 3) and 0.4% (Table 4) respectively. There was a statistically significant difference (p=0.02) between the prevalence of *Cryptosporidium* species antigen in sheep and the age of the animals sampled. The overall prevalence of *Cryptosporidium* species antigen in dog faeces was 1.1% (1/90) (Table 5). However, a significantly higher prevalence of *Cryptosporidium* species antigen (p=0.05) was observed in dogs passing out loose faeces (5.3%) than in those with firmly formed faeces (0.0%).

All the 15 (10 for cattle, 3 for sheep, 1 for goat, 1 for dogs) faecal samples that were positive for *Cryptosporidium* using ELISA screening, generated 18S rRNA PCR products with the expected band of approximately 830 bp (Plate I). DNA sequencing of the 18S rRNA PCR products from positive samples confirmed the identification of the *Cryptosporidium* and detected the species. One positive sample from the PCR product could not be sequenced, thus failed quality control. The partial 18S rRNA gene sequences obtained from the fourteen products had above 98% similarity to reference sequences downloaded from the GenBank for *C. hominis*, *C. parvum, C. andersoni*, *C. muris* (Table 6). Although, among species detected, *C. andersoni* (42.9%) was the most prevalent species followed by *C. muris* (21.4%) and *C. parvum* (21.4%), the least was *C. hominis* (14.3%). Table 7 shows the distribution of *Cryptosporidium* species (GenBank accession numbers: OQ605420-605433,

Table 1: PCR Primers and amplicon sizes

Target gene	Primer name	Primer sequence (5' to 3')	Amplicon size (bp)	References
18S rRNA	F1:LX0697	AACCTGGTTGATCCTGCCAGTAGTC	1,325	Xiao *et al*. (2000)
	R1:LX0669	TGATCCTTCTGCAGGTTCACCTACG		
18S rRNA	F2:LX0698	GGAAGGGTTGTATTTATTAGATAAAG	830	Xiao *et al*. (2000)
	R2:LX0670	CTCATAAGGTGCTGAAGGAGTA		

Table 2: Prevalence of *Cryptosporidium* species antigen in cattle faeces in sedentary Fulani herds in selected Local Government Areas of Kaduna State, Nigeria (n = 240)

Variables	Category	Number Examined	Number positive	Specific rate (%)	χ^2/Fishers exact	p-value
Age (years)	≤1	83	3	3.6	0.097	0.76
	> 1	157	7	4.5		
Sex	Male	98	3	3.1	0.507	0.48
	Female	142	7	4.9		
Breed	White Fulani	190	8	4.2	0.227	0.89
	Sokoto Gudali	45	2	4.4		
	Red Bororo	5	0	0.0		
Body Condition Score	Poor	65	3	4.6	3.865	0.15
	Medium	63	5	7.9		
	Good	112	2	1.8		
Consistency of faeces	Loose	55	1	1.8	0.986	0.32
	Firmly-formed	185	9	4.9		
LGAs	Zaria	40	2	5.0	5.843	0.32
	Sabon Gari	40	3	7.5		
	Giwa	40	3	7.5		
	Igabi	40	0	0.0		
	Soba	40	0	0.0		
	Kudan	40	2	5.0		
Total		240	10	4.2		

Table 3: Prevalence of *Cryptosporidium* species antigen in sheep faeces in sedentary Fulani herds in selected Local Government Areas of Kaduna State, Nigeria (n = 180)

Variables	Category	Number Examined	Number positive	Specific rate (%)	χ^2/Fishers exact	p-value
Age (months)	≤ 6	62	3	4.8	5.806	0.02
	> 6	118	0	0.0		
Sex	Male	64	0	0.0	1.683	0.19
	Female	116	3	2.6		
Breed	Yankasa	138	3	2.2	0.929	0.63
	Balami	39	0	0.0		
	Uda	3	0	0.0		
Body Condition Score	Poor	54	1	1.9	3.272	0.20
	Medium	47	2	4.3		
	Good	79	0	0.0		
Consistency of faeces	Loose	41	0	0.0	0.900	0.34
	Firmly-formed	139	3	2.2		
LGAs	Zaria	30	1	3.3	3.05	0.69
	Sabon Gari	30	1	3.3		
	Giwa	30	0	0.0		
	Igabi	30	0	0.0		
	Soba	30	1	3.3		
	Kudan	30	0	0.0		
Total		180	3	1.7		

Table 4: Prevalence of *Cryptosporidium* species antigen in goat faeces in sedentary Fulani herds in selected Local Government Areas of Kaduna State, Nigeria (n = 240)

Variables	Category	Number Examined	Number positive	Specific rate (%)	χ^2/Fishers exact	p-value
Age (months)	≤ 6	81	1	1.2	1.971	0.16
	> 6	159	0	0.0		
Sex	Male	100	1	1.0	1.406	0.24
	Female	140	0	0.0		
Breed	Kano brown	188	1	0.5	0.278	0.87
	Red Sokoto	47	0	0.0		
	West African dwarf	5	0	0.0		
Body Condition Score	Poor	67	1	1.5	2.59	0.27
	Medium	61	0	0.0		
	Good	112	0	0.0		
Consistency of faeces	Loose	58	1	1.7	3.151	0.08
	Firmly-formed	182	0	0.0		
LGAs	Zaria	40	0	0.0	5.021	0.41
	Sabon Gari	40	1	2.5		
	Giwa	40	0	0.0		
	Igabi	40	0	0.0		
	Soba	40	0	0.0		
	Kudan	40	0	0.0		
Total		**240**	**1**	**0.4**		

Table 5: Prevalence of *Cryptosporidium* species antigen in dog faeces in sedentary Fulani herds in selected Local Government Areas of Kaduna State, Nigeria (n = 90)

Variables	Category	Number Examined	Number positive	Specific rate (%)	χ^2/Fishers exact	p-value
Age (months)	≤ 6	29	1	3.4	2.127	0.15
	> 6	61	0	0.0		
Sex	Male	64	1	1.6	0.411	0.52
	Female	26	0	0.0		
Breed	Local	87	1	1.1	0.035	0.85
	Crossbreed	3	0	0.0		
Body Condition Score	Poor	30	0	0.0	3.323	0.19
	Medium	21	1	4.8		
	Good	39	0	0.0		
Consistency of faeces	Loose	19	1	5.3	3.779	0.05
	Firmly-formed	71	0	0.0		
LGAs	Zaria	15	0	0.0	5.056	0.41
	Sabon Gari	15	0	0.0		
	Giwa	15	1	6.7		
	Igabi	15	0	0.0		
	Soba	15	0	0.0		
	Kudan	15	0	0.0		
Total		**90**	**1**	**1.1**		

NCBI (2023) and the risk factors among cattle, sheep, goats and dogs in sedentary herds in selected LGAs of Kaduna State, Nigeria. The phylogenetic tree, constructed using sequences obtained from isolates from cattle, sheep, goats, and dog samples, showed three distinct groups (clades); the first clade (bootstrap value= 72%) comprising of *C. hominis* and *C. parvum*, the second clade (bootstrap value= 72%), *C. andersoni* and the third clade (bootstrap value= 67%), *C. muris* (Figure 1).

Table 6: Distribution of *Cryptosporidium* species among cattle, sheep, goats and dogs in sedentary Fulani herds in selected Local Government Areas (LGAs) of Kaduna State, Nigeria

Animal spp.	*Cryptosporidium* spp.	*C. hominis*	*C. parvum*	*C. muris*	*C. andersoni*
Cattle	9	2	3	2	2
Sheep	3	0	0	0	3
Goat	1	0	0	0	1
Dogs	1	0	0	1	0
Total	14	2 (14.3%)	3 (21.4%)	3 (21.4%)	6 (42.9%)

Table 7: Distribution of *Cryptosporidium* species with possible risk factors among cattle, sheep, goats and dogs in sedentary Fulani herds in selected Local Government Areas (LGAs) of Kaduna State, Nigeria

Isolate No.	Animal spp	Crypto spp.	Accession no.	Age	Sex	Breed	BCS	Faeces	LGA
C52	C	*C. parvum*	OQ605420	>1 year	F	White Fulani	Md	FF	Zaria
C54	C	*C. hominis*	OQ605433	>1 year	F	White Fulani	Md	FF	Zaria
C140	C	*C. muris*	OQ605423	>1 year	F	Sokoto Gudali	Gd	FF	Kudan
C149	C	*C. parvum*	OQ605429	0-1 year	M	Sokoto Gudali	Md	FF	Kudan
C169	C	*C. parvum*	OQ605427	0-1 year	M	White Fulani	Pr	FF	Giwa
C183	C	*C. andersoni*	OQ605424	>1 year	F	White Fulani	Md	FF	Giwa
C192	C	*C. hominis*	OQ605426	0-1 year	F	White Fulani	Pr	LF	Giwa
C209	C	*C. muris*	OQ605425	>1 year	F	White Fulani	Gd	FF	Sabon gari
C214	C	*C. andersoni*	OQ605422	>1 year	M	White Fulani	Md	FF	Sabon gari
S49	S	*C. andersoni*	OQ605428	0-6 months	F	Yankasa	Md	FF	Zaria
S64	S	*C. andersoni*	OQ605430	0-6 months	F	Yankasa	Pr	FF	Soba
S156	S	*C. andersoni*	OQ605421	0-6 months	F	Yankasa	Md	FF	Sabon gari
G228	G	*C. andersoni*	OQ605432	0-6 months	M	Kano brown	Pr	LF	Sabon gari
D65	D	*C. muris*	OQ605431	0-6 months	M	Local	Md	LF	Giwa

C=cattle, S=sheep, G=goat, D=dog; F=female, M=male; Gd=good, Md=medium, Pr=poor; FF= Firmly-formed, LF=loosely formed

Discussion

The prevalence of *Cryptosporidium* coproantigens in cattle observed in this study is lower than the 32.3% prevalence in Oyo State, Nigeria reported by Ayinmode & Fagbemi (2011) and lower than the reports in other parts of the world: 11% of calves in Sweden (Bjorkman *et al*., 2003), 14% of cows in Denmark (Maddox-Hyttel *et al*., 2006) and 19.7% of cows in Tanzania (Swai *et al*., 2007). This observation may be associated with the low rainfall and relative humidity characteristic of the northern part of the country which affects the survival of the oocysts in the environment (Wang *et al*., 2023). The prevalence of *Cryptosporidium* coproantigens in sheep reported

in this study is lower than 22.2% prevalence in Ethiopia (Regassa *et al*., 2013) and 40% prevalence reported in a University Teaching farm in Nigeria (Akinkuotu *et al*., 2014). The prevalence of *Cryptosporidium* coproantigens in goats observed in this study was lower than 24.0% in Plateau State and 43.9% in Ogun State, previously reported by Pam *et al*. (2013) and Akinkuotu *et al*. (2014) respectively. It may also be attributed to the ecological and environmental characteristics of the study area which is in contrast to the high relative humidity and long periods of rainfall of the southern regions of the country, thus this study suggests that the prevalence increases as one moves from north to south. The differences in the prevalence of *Cryptosporidium* infections in sheep and goats in different regions may be due to the differences in the levels of contamination of the environment with viable oocysts of the parasite which strive more in wet and humid regions. The higher prevalence of *Cryptosporidium* infection in younger animals as compared to the adults may be because calves are less immune to infection as immunity acquired during birth from the dams gradually wanes off making the calves vulnerable to infections (Bartley & Katzer, 2015).

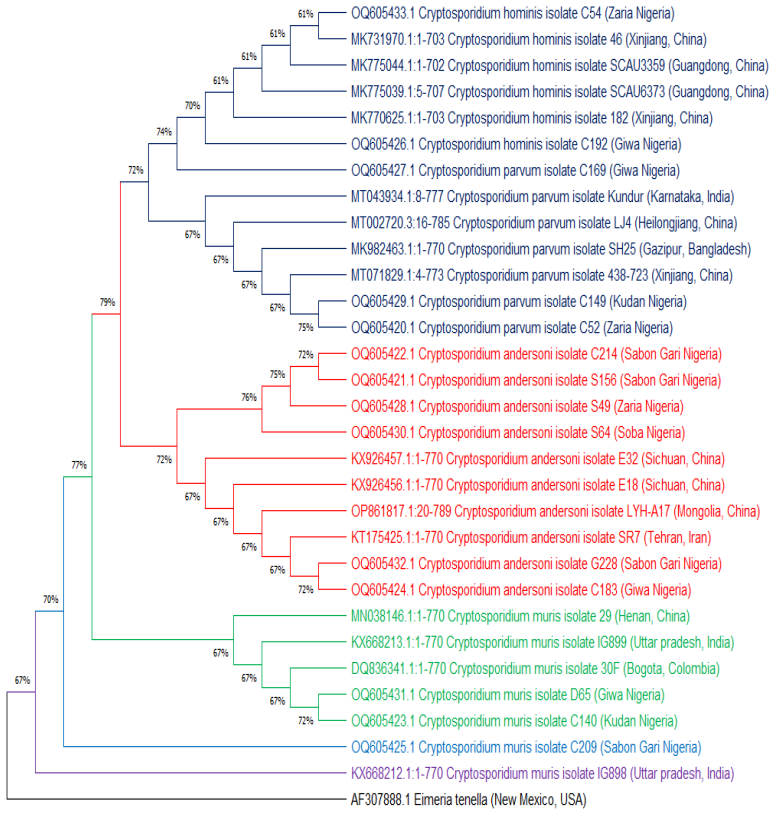

Figure 1: Maximum composite likelihood phylogenetic tree based on the 18 SSU rRNA gene of *Cryptosporidium* spp. in isolates from cattle, sheep, goats and dogs in sedentary Fulani herds in selected LGAs of Kaduna State, Nigeria, with selected reference sequences of *Cryptosporidium* spp. and *Eimeria tenella* from the GenBank, using Maximum Likelihood method of ClustalW2-Phylogeny

Higher infection rates were observed in dogs with loose/watery faeces than those passing out firmly formed faeces. This observation is in agreement with the works of Olabanji *et al*. (2016). It could be due to *Cryptosporidium* oocysts inhabiting the inner lining of the small intestine, where excystation takes place releasing motile trophozoites and due to the migratory habits of these trophozoites along the villi of the small intestine, there is irritation, and sloughing off of the lining of the intestine which is seen as diarrhoea.

Results also showed that *C. andersoni* was the most prevalent species identified in this study. This finding is in agreement with the results of previous studies done on dairy cattle by Kvac *et al*. (2004) who stated that *C. andersoni* infects cattle of all ages. Thus, the presence of *C. andersoni* in this study could be a result of the wide age groups (calves and adults) sampled. Reports show that the majority of *C. parvum* infections appear to be limited to dairy calves under eight weeks of age (Fayer *et al*., 2007). It is therefore not surprising that *C. parvum* was detected in calves ≤1year in this study, as it is a dominant parasite in pre-weaned dairy calves (Fayer *et al*., 2007). Also, *C. parvum* and *C. andersoni* identified in this study are among the four major species in addition to *C. bovis* and *C. ryanae* in cattle that have been reported worldwide (Abdelaziz *et al*., 2022). This finding is slightly different from the report of Okojokwu *et al*. (2016b), who also isolated *C. parvum*, in addition to *C. bovis* and *C. ryanae* from cattle slaughtered in abattoirs in Kaduna State; Maikai *et al*. (2011), who also reported *C. andersoni*, plus *C. bovis* and *C. ryanae*

in calves within Kaduna State; Ayinmode and Fagbemi (2010) did not detect *C. parvum* and *C. andersoni* in calves in Oyo State, Nigeria. These disparities could be due to the ages of animals sampled, systems of management, the season of the year during which the study was done and the geographical location of the study. The occurrence of *C. andersoni* in both sheep and goats in this study is in agreement with the report of Xiao (2010) and Yang *et al*. (2014) and this could be due to the practice of grazing sheep along with cattle in marshy areas, increasing their chances of exposure to the parasite. Although the goats were not grazed with cattle in the field, this study observed that they were housed close to the cattle herds in sedentary settings, thereby increasing the chances of cross-infection. Although, *C. hominis* in humans has long been known to have a much narrower host range than the morphologically similar *C. parvum*, (Morgan-Ryan *et al*., 2002) but the finding of *C. hominis* in cattle sampled in the course of this study is consistent with reports of Wang *et al*. (2011) and Chen & Huang (2012) who reported the occurrence of *C. hominis* in dairy calves in China. Humans are regarded as the major host of *C. hominis,* but sporadic natural infection can result due to close contact of humans and these domestic animals.

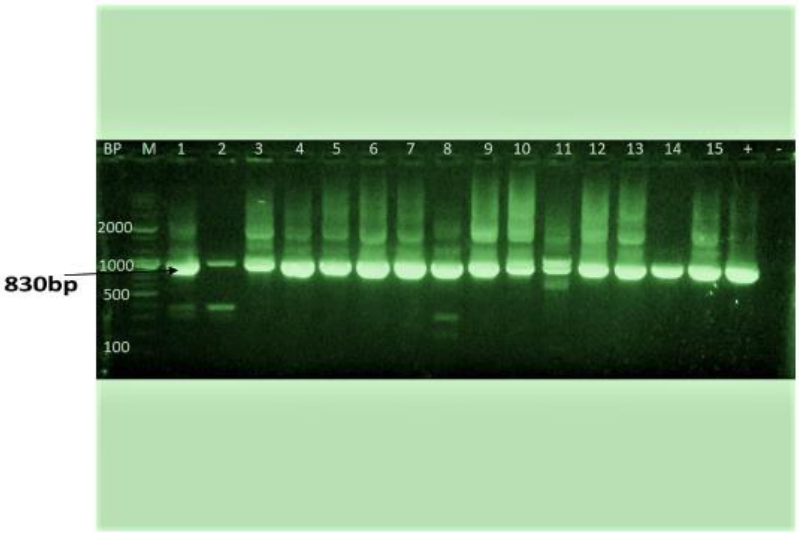

Plate I: Electrophoretic (1.5% agarose) separation of the amplicon of 18S rRNA gene fragment. Lane M: 100 bp ladder (Marker); lane +: positive control (*C. parvum*); lane -: negative control (master mix) lane 1-15: isolates (C52, C204, S156, C214, C140, C183, C209, C192, C169, S49, C149, S64, D65, G228, C54)

This study reported the occurrence of *C. muris* in cattle which was also reported by Nakai *et al*. (2004) in Japan. Rodents which are the major hosts for *C. muris* were found around these sedentary herds, and they play a role in the faecal contamination of shrubs, grasses and water sources with infective oocysts, increasing the possibility of infection in cattle. The occurrence of *C. muris* in guard/hunting dogs sampled in this study is in conformity with the reports of Philip *et al*. (2008) in Texas and that of Yoshiuchi *et al*. (2010) in Japan. This finding could be due to the scavenging habit of these dogs in search of food in refuse dumps and cow dung, increasing chances of infection. Also, the hunting and consumption of rodents which could be harbouring the developing stages of the parasite, by the dogs could be a source of infection.

An age-related distribution of *C. parvum* and *C. andersoni* observed in this study has also been reported in dairy cattle (Fayer *et al*., 2007; Feng *et al*., 2007). The majority of the *Cryptosporidium* species detected in this study were from female animals which could be due to stress of hormonal imbalance during pregnancy and lactation which is common among dairy animals (Olson *et al*., 2004). The SSU amplicons of the 18S rRNA gene that were sequenced from 11 of the isolates were from animals with firmly-formed faeces, but it should be noted that *C. andersoni* infections are usually not associated with diarrhoeal disease in dairy cattle (Fayer *et al*., 2007). This study observed that isolates positive for *Cryptosporidium* were from animals with either medium or poor body condition scores. This could be because *C. andersoni* being the dominant species in this study is known to be associated with gastritis, reduced milk yield (in dams), poor weight gain (in calves mostly) (Huang *et al*., 2014) thus, low body condition score.

The phylogenetic tree drawn using sequences obtained from the isolates showed that *Cryptosporidium* species identified in this study have an evolutionary relationship, using the bootstrap value guide by Tamura *et al*. (2021) and there was minimal sequence divergence. This tree also shows there is an evolutionary relationship between *Cryptosporidium* species from these selected LGAs and *Cryptosporidium* species isolated from China, India, Iran, Colombia, and Bangladesh. This study shows that *C. hominis* and *C. parvum* are more closely related, which was also reported by Morgan-Ryan *et al*. (2002) who stated that *C. hominis* is

morphologically similar *C. parvum*. The potential zoonotic transmission of *C. andersoni* and *C. muris* is unknown, but the species have been isolated from humans with diarrhoea (Leoni *et al*., 2006). The presence of *C. parvum* and *C. hominis* in cattle in this study suggests that cattle in these LGAs have a high potential for the transmission of *Cryptosporidium* to humans.

Cryptosporidiosis is an important protozoan disease and one of the debilitating neglected diseases of livestock in Nigeria, causing direct and indirect losses in cattle production in different parts of the country. We therefore conclude that, as *C. andersoni* appears to be the most prominent species among those detected, further research should investigate its specific transmission routes and reservoir hosts to develop more targeted control strategies. Addressing the identified risk factors associated with *Cryptosporidium* infection in different animal species in this study is crucial and implementing targeted interventions, such as vaccination programs and improved animal husbandry practices, could help mitigate these risks.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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